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COMPLEX IMMUNIZATION OF ANIMALS AGAINST  
ANTHRAX AND BRUCELLOSIS

COUNTRY: USSR

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FSTC-HT-23-547-68

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ANTHRAX AND BRUCELLOSIS

by

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Source: VETERINARIYYA  
(Veterinary Science)  
No. 7, pp 45-47, 1967  
USSR

Translated for FSTC by Techtran Corporation

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UNCLASSIFIED  
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Foreign Science and Technology Center US Army Materiel Command Department of the Army	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED
2b. GROUP	

3. REPORT TITLE

COMPLEX IMMUNIZATION OF ANIMALS AGAINST ANTHRAX AND  
BRUCELLOSIS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

- Translation

5. AUTHOR(S) (First name, middle initial, last name)

A. B. Boyakhchyan and P.L. Kalashyan

6. REPORT DATE 22 April 1969	7a. TOTAL NO. OF PAGES 5	7b. NO. OF REFS N/A
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S)	
8c. PROJECT NO. e. 2301 9223628	8d. OTHER REPORT NO(S) (Any other numbers that may be assigned to report) ACSI Control Number (None)	
8e. SMUFD-PR-I		

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY US Army Foreign Science and Technology Center
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13. ABSTRACT

The feasibility of simultaneous immunization of animals against anthrax and brucellosis has been studied in guinea pigs and sheep. Complex vaccination did not suppress the immunobiological reactions characteristic of these infections, and the thermal reaction was more pronounced than upon separate vaccinations. Upon complex vaccination, the periods of appearance, maximal development, and disappearance of the allergic and thermoallergic reactions of anthrax and brucellosis were indistinguishable from those observed after separate immunizations. Simultaneous injection of anthrax and brucellosis antigens not only does not suppress development of allergies to the corresponding diseases, but even somewhat stimulates these processes. Upon controlled infection with a lethal dose of anthrax culture 5.5 months after immunization by the complex method, sheep demonstrated resistance.

**UNCLASSIFIED**  
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
complex immunization anthrax brucellosis immunology						

**UNCLASSIFIED**  
Security Classification

## COMPLEX IMMUNIZATION OF ANIMALS AGAINST ANTHRAX AND BRUCELLOSIS

The purpose of our research is to study the feasibility of a single immunization of animals against anthrax and brucellosis. The work was carried out on guinea pigs and sheep. For immunization of the animals against anthrax, an aluminum hydroxide vaccine of the GNKI was used, and a brucellosis vaccine from strain 19 was used against brucellosis.

Three groups of guinea pigs (about 10 guinea pigs in each) were selected in the experiment. Animals of the first group were immunized against anthrax; of the second group, against brucellosis, and of the third, simultaneously against anthrax and brucellosis.

Table 1  
Results of Allergic Reaction

a. Группа	b. Число животных	c. Вакцина	d. Дни появления реакции	Положительно реагировали e. (по часам)			
				0	24	36	48
Контроль f.	5	g. Сибиряземная	10	4	4	2	1
	4	10.16	—	—	—	—	—
	5	13 <sup>o</sup>	4	4	3	3	2
	5	30	3	3	3	3	2
	4	48	3	3	3	3	3
	5	40 <sup>**</sup>	2	4	4	3	3
g. 13-й день избран как средний срок по отношению к 10-му и 16-му дням.							
h. 40-й день является средним между 30-м и 40-м днями.							

a. Group b. Number of guinea pigs c. Vaccine d. Days of formation of reaction e. Reacted positively (In Hours) f. Control g. Anthrax	h. Anthrax + brucellosis i. Brucellosis j. 13th day selected as average period in relation to 10th and 16th days. k. 40th day is mean between 30th and 40th days
--	---

The immunobiological rearrangement of the organisms of the animals tested of the first group was confirmed by means of establishment of an allergic sample with the help of anthraxine by the method of Shlyakhov, which suggests the use of anthraxine for determination of the intensity of anthrax immunity in people. The indicated allergen was injected subcutaneously in a dose of 0.1 ml at two times: 10 and 16 days after immunization. The reaction was read after 48 hours. A reaction was considered to be positive when a diffuse edematous tumescence 2x3 cm in dimensions was observed in the animals at the site of injection of the preparation. In eight guinea pigs the allergic reaction was positive (Table 1).

In all of the determinations of the possibility of an effect of the brucellosis vaccine on the sensitization of the guinea pig organisms to anthrax antigen, five guinea pigs from the third group were subjected to anthraxinization on the 13th day after the combined immunization. Four guinea pigs reacted positively.

In order to confirm the allergic state of the animals immunized against brucellosis by the combined vaccine against the two diseases, guinea pigs of the second group (30th and 48th days) and the third (40th day) were studied by brucello-hydrolysate. Of the nine live guinea pigs remaining of the second group, six had a positive reaction, and out of the five animals of the third group, four reacted.

Since there is no single opinion on the formation of antibodies during positive allergic reactions, we have also studied indications of agglutination reaction, in guinea pigs of the second and third groups (Table 2). It has been established that on the 30th day after immunization a positive reaction appeared at a titer of 1:80 in nine guinea pigs of the second group and on the 60th day in four, and out of 10 guinea pigs of the third group on the 30th day nine reacted and on the 60th day, three.

By bacteriological research on 15 guinea pigs of the second and third groups killed on the 63rd day, a pure Brucella culture was isolated in only one (from the second group).

The research carried out indicates that simultaneous injection of guinea pigs with anthrax and brucellosis live vaccines does not hinder the development of immunobiological reactions characteristic of anthrax and brucellosis.

Table 2  
Results of Investigation of Guinea Pigs  
for Agglutination Reaction

a. Группа	b. Число исследуемых свиней	c. Дни исследования	d. Положительная реакция (два-четыре креста)		
			13:00	e. Максимальное разведение	
				1:320	1:640
II	9 10	13:00 13:00	9 4 9 3	5 — 5 1	— — 2 1
III					

a. Group

d. Positive reaction (2-4+)

b. Number of guinea pigs

e. Maximum attenuation

c. Days of investigation

For simultaneous vaccination the period of appearance and disappearance, and also the intensity of the allergic and serological reaction was almost indistinguishable from that observed upon vaccination of guinea pigs against anthrax and brucellosis separately.

Experiments in sheep were carried out in 1965 at the Kirov Collective Farm in the Stepanavan region. 114 sheep (3 groups) were taken for the experiment. 24 sheep of the first group were immunized against anthrax, 12 of the second group were immunized against brucellosis, and 54 sheep of the third group were immunized simultaneously at various sites against both of the indicated diseases, 24 sheep served as control. Data of thermal, thermoallergic, allergic, and serological research served as the criteria for immunobiological reactivity.

The research indicated that the temperature reaction in the animals immunized by the complex method does not differ from the reaction upon separate immunization, and on the average even exceeded it by 0.3-0.4°.

Multiple research on the blood of immunized sheep of the second and third groups according to agglutination reactions and complement fixation tests indicated that simultaneous injection of the animals with vaccines against the two diseases does not suppress the process of formation of immunity against brucellosis (Table 3). In essence, a difference was not established in the indicators of these reactions in sheep of different groups. These data completely refute the opinion of several investigators on

suppression of the immunobiological reaction under the influence of anthrax antigen.

Table 3  
Indicators of Positive CA and CFT in Sheep of the  
2nd and 3rd Groups (about 12 Animals in each)  
In Five Investigations

a. Разведение	b. Первое			c. Второе			d. Третье			e. Четвертое			f. Пятое		
	g. RA		h. РСК	g. RA		h. РСК	g. RA		h. РСК	g. RA		h. РСК	g. RA		h. РСК
	II	III		II	III		II	III		II	III		II	III	
1:3200	3	1	—	2	2	—	1	4	—	—	—	—	—	—	—
1:1600	9	8	—	6	6	—	2	6	—	—	—	—	—	—	—
1:800	7	—	—	9	9	—	4	9	—	—	—	—	—	—	—
1:400	11	10	—	11	11	—	10	11	—	—	—	—	—	—	—
1:200	11	11	—	11	11	—	11	11	—	—	—	—	—	—	—
1:100	12	12	—	12	12	—	12	12	—	—	3	—	2	—	—
1:50	12	12	—	12	12	—	12	12	—	7	4	—	2	4	—
1:25	12	12	—	12	12	—	12	12	—	—	—	—	2	4	—
1:12.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:6.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:3.125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:1.5625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.78125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.390625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.1953125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.09765625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.048828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0244140625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.01220703125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.006103515625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0030517578125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00152587890625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000762939453125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0003814697265625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00019073486328125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000095367431640625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000476837158203125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00002384185791015625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000011920928955078125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000059604644775390625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000298023223876953125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000001490116119384765625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000007450580596923828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000037252902984691440625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000186264514922457203125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000931322574611286015625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000004656612873056430078125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000023283064365282150390625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000116415321826410751953125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000000582076609132053759765625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000002910383045660268798828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000000145519152283013439940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000007275957614150671997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000003637978807075335998828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000000181898900353766799940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000000009094945017688339997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000000004547472508844169998828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000227373625442208999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000000011368681272110499997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000056843406360552499998828125	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000028421703180276249999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000014210851590138124999997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.00000000000071054257950690612499999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000003552712897534531249999997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000001776356448767265624999999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000000888178224383632812499999997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000000444089112191816406249999999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.0000000000000222044556059508203124999999997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
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1:0.000000000000002775556950743810624999999999940625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1:0.000000000000001387778475371950312499999999997590625	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
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and by calculation of the difference in temperature after measuring it 12 times in the course of one day.

The data obtained indicates the absence of a difference in the indicators of temperature in sheep of the three groups. Thus, the difference in temperature after injection of brucellosis vaccine in sheep of the second group according to the times consisted of 1.5 and 1.6° and in sheep of the third group - 1.5 and 1.4°. After injection of anthrax vaccine in sheep of the first group the difference in temperature was 1.7 and 1.6°, and in animals of the third group was 1.5 and 1.6° respectively.

In all of the definitive decisions on the effectiveness of complex immunization, as a control about four sheep from the first and third groups were infected after 5.5 months with two control animals. The animals were infected twice with a lethal dose of the virulent strain No. 147 of anthrax culture. Experimental sheep demonstrated resistance to this strain. In three sheep a brief increase in temperature was noted. Control sheep died from anthrax: one, on the third and the others, on the fourth day after infection.

#### Conclusions

1. Simultaneous injection of live anthrax and brucellosis vaccines to guinea pigs and sheep did not suppress the development of the immunobiological reactions characteristic for the indicated infections.
2. After complex vaccination, the thermal reaction in animals was more pronounced than after separate vaccinations.
3. Upon complex vaccination the time of appearance, maximal development, and disappearance of the allergic and thermoallergic reactions in anthrax and brucellosis were not distinguishable from those after separate immunizations.
4. Simultaneous injection of anthrax and brucellosis antigens not only does not suppress development of allergy to the corresponding infections, but even somewhat stimulates these processes.
5. Sheep immunized by the complex method upon control infection with a lethal dose of anthrax culture after 5.5 months, demonstrated resistance.